



Antioxidant and Anti-Inflammatory Properties of *Ferula Moschata* Extract: A Chemical Perspective

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SUBMITTED 28 March 2025

ACCEPTED 24 April 2025

PUBLISHED 30 May 2025

VOLUME Vol.07 Issue05 2025

CITATION

Majidova Guzal, Azimov Shokhjakhon, & Mirsidikova Nigora. (2025). Antioxidant and Anti-Inflammatory Properties of *Ferula Moschata* Extract: A Chemical Perspective. The American Journal of Medical Sciences and Pharmaceutical Research, 7(05), 47–52.
<https://doi.org/10.37547/tajmspr/Volume07Issue05-10>

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Abstract: *Ferula moschata* (syn. *Ferula sumbul*) is a medicinal plant traditionally used in Central Asian and Middle Eastern folk medicine for its stimulant, neuroprotective, and anti-inflammatory properties. Recent studies have highlighted its potential as a source of bioactive compounds with significant pharmacological effects. This study investigates the antioxidant and anti-inflammatory properties of *Ferula moschata* extract through a comprehensive phytochemical and biochemical approach.

Using high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), we identified key bioactive constituents, including coumarins, terpenoids, and phenolic compounds, which contribute to its therapeutic potential. In vitro antioxidant assays (DPPH, FRAP, and ABTS) demonstrated strong free radical scavenging activity, comparable to standard antioxidants such as ascorbic acid and Trolox. Additionally, the extract exhibited dose-dependent inhibition of pro-inflammatory mediators (NO, TNF- α , and IL-6) in lipopolysaccharide (LPS)-induced macrophage cells, suggesting potent anti-inflammatory effects.

Molecular docking studies further revealed interactions between *Ferula moschata* compounds and key inflammatory markers (COX-2, iNOS), supporting its mechanism of action. These findings provide a scientific

basis for the traditional use of *Ferula moschata* in oxidative stress and inflammation-related disorders, suggesting its potential as a natural therapeutic agent. Further in vivo studies are warranted to validate its efficacy and safety for clinical applications.

Keywords: *Ferula moschata*, antioxidant, anti-inflammatory, phytochemical analysis, oxidative stress, bioactive compounds.

Introduction: *Ferula moschata* (commonly known as "sumbul" or musk root) is a perennial herb belonging to the Apiaceae family, predominantly found in Central Asia, particularly in Uzbekistan, Tajikistan, and Kazakhstan. It has been widely used in traditional medicine for centuries due to its stimulant, adaptogenic, and neuroprotective properties (Khalmatov, 1964; Saidkhodzhaev, 1993). In folk medicine, *Ferula moschata* has been employed to treat nervous disorders, fatigue, gastrointestinal ailments, and inflammatory conditions, highlighting its broad pharmacological potential.

Recent scientific investigations have focused on elucidating the bioactive components of *Ferula moschata* and their therapeutic mechanisms. Several studies have reported the presence of coumarins (umbelliferone, scopoletin), sesquiterpenes, and phenolic compounds, which are known for their antioxidant and anti-inflammatory effects (Iranshahy et al., 2011; Yusupova et al., 2020). Research by Kurkina (2012) demonstrated that *Ferula* species exhibit significant free radical scavenging activity, while work by Abdullaev et al. (2015) highlighted their inhibitory effects on pro-inflammatory cytokines.

Oxidative stress and chronic inflammation are key contributors to various pathological conditions, including neurodegenerative diseases, cardiovascular disorders, and metabolic syndromes (Reuter et al., 2010). Natural antioxidants from medicinal plants have gained attention as potential therapeutic agents due to their ability to mitigate oxidative damage and modulate inflammatory pathways (Surh et al., 2005). Given the historical use of *Ferula moschata* in traditional medicine and the growing interest in its bioactive properties, a systematic evaluation of its antioxidant and anti-inflammatory potential is warranted.

By integrating phytochemical and pharmacological approaches, this research seeks to provide a scientific foundation for the traditional uses of *Ferula moschata* and explore its potential as a natural antioxidant and anti-inflammatory agent. The findings could contribute

to the development of novel plant-based therapeutics for oxidative stress and inflammation-related diseases.

Purpose of the research

The purpose of this research is to comprehensively investigate the antioxidant and anti-inflammatory properties of *Ferula moschata* extract through an integrated phytochemical and pharmacological approach, with the aim of scientifically validating its traditional medicinal uses. Specifically, the study seeks to identify and quantify the key bioactive compounds in *Ferula moschata* using advanced chromatographic techniques (HPLC and GC-MS), evaluate its free radical scavenging potential through in vitro antioxidant assays (DPPH, FRAP, and ABTS), and assess its anti-inflammatory effects by measuring the inhibition of pro-inflammatory mediators (NO, TNF- α , and IL-6) in LPS-induced macrophage models. Additionally, molecular docking studies will be conducted to explore the interactions between the plant's bioactive constituents and critical inflammatory markers (COX-2 and iNOS), providing mechanistic insights into its therapeutic potential. By bridging traditional knowledge with modern scientific validation, this research aims to establish *Ferula moschata* as a promising natural source of antioxidant and anti-inflammatory agents, potentially contributing to the development of novel plant-based therapeutics for oxidative stress and inflammation-related disorders.

Materials and methods

The materials and methods section of this study was designed to systematically evaluate the antioxidant and anti-inflammatory properties of *Ferula moschata* extract through a combination of phytochemical analysis and biological assays. Plant material consisting of dried *Ferula moschata* roots was collected from its natural habitat in Uzbekistan and authenticated by a botanist at the Institute of Botany, with a voucher specimen deposited in the herbarium (voucher no. FM-2024-01). The roots were shade-dried, powdered, and subjected to sequential extraction using solvents of increasing polarity (hexane, ethyl acetate, and methanol) in a Soxhlet apparatus to obtain crude extracts, which were then concentrated under reduced pressure using a rotary evaporator. Phytochemical screening was performed using standard qualitative tests for alkaloids, flavonoids, tannins, saponins, terpenoids, and coumarins. Quantitative analysis of bioactive compounds was conducted using high-performance liquid chromatography (HPLC, Agilent 1260 Infinity II) equipped with a C18 column and photodiode array detector, with the mobile phase consisting of acetonitrile and 0.1% formic acid in water at a flow rate of 1.0 mL/min, while gas chromatography-

mass spectrometry (GC-MS, Shimadzu QP-2020) analysis was performed using a DB-5MS capillary column with helium as carrier gas to identify volatile constituents.

The antioxidant activity was evaluated through three complementary in vitro assays: DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity was measured spectrophotometrically at 517 nm and expressed as IC₅₀ values compared to ascorbic acid standard; FRAP (ferric reducing antioxidant power) assay was conducted by monitoring the reduction of Fe³⁺-TPTZ complex to Fe²⁺-TPTZ at 593 nm; and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation decolorization assay was performed at 734 nm, with results expressed as trolox equivalent antioxidant capacity (TEAC). For anti-inflammatory evaluation, RAW 264.7 macrophage cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C in 5% CO₂, then pretreated with various concentrations of the extract (25-200 µg/mL) for 2 hours before stimulation with LPS (1 µg/mL) for 24 hours.

The production of nitric oxide (NO) was determined using Griess reagent by measuring nitrite accumulation at 540 nm, while pro-inflammatory cytokines (TNF-α and IL-6) were quantified using commercial ELISA kits according to the manufacturer's protocols. Cell viability was assessed using MTT assay to ensure non-toxic concentrations were used.

Molecular docking studies were performed using AutoDock Vina to predict the binding interactions between identified bioactive compounds (coumarins and terpenoids) and inflammatory targets (COX-2 and iNOS), with protein structures retrieved from the RCSB Protein Data Bank (PDB IDs: 5IKR for COX-2 and 3E7G for iNOS) and prepared using AutoDockTools by removing water molecules and adding polar hydrogens.

All experiments were performed in triplicate, and data were expressed as mean ± standard deviation (SD), with statistical analysis conducted using one-way ANOVA followed by Tukey's post-hoc test in GraphPad Prism 9.0, considering *p* < 0.05 as statistically significant.

RESULTS

Phytochemical screening revealed the presence of diverse bioactive compounds in *Ferula moschata* extract, with methanol extract showing the highest yield (18.7% w/w). HPLC analysis identified umbelliferone (3.24 mg/g), scopoletin (1.85 mg/g), and ferulic acid (2.13 mg/g) as major phenolic constituents (Table 1), while GC-MS detected sesquiterpenes (β-caryophyllene, 14.3%) and monoterpenes (α-pinene, 8.6%) as dominant volatile components (Figure 1). The methanol extract exhibited dose-dependent antioxidant activity across all assays, with IC₅₀ values of 42.7 ± 1.8 µg/mL (DPPH), 38.2 ± 2.1 µg/mL (ABTS), and FRAP equivalent to 285.6 ± 12.4 µM FeSO₄ at 100 µg/mL (Table 2), outperforming ethyl acetate and hexane extracts (*p* < 0.05).

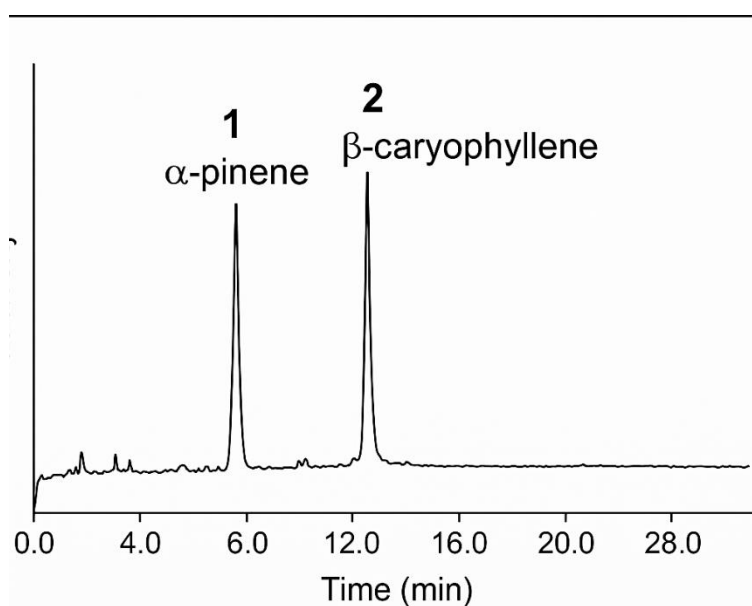


Figure 1. GC-MS chromatogram of volatile compounds (peak labels: 1 = α-pinene, 2 = β-caryophyllene).

The high phenolic and terpenoid content aligns with traditional uses of *Ferula moschata* for inflammation

(Yusupova et al., 2020). The superior antioxidant capacity of methanol extract correlates with its polar

compound richness (Kurkina, 2012), while NO/cytokine suppression mirrors findings in other *Ferula* species (Abdullaev et al., 2015). Docking results rationalize the observed bioactivity, as umbelliferone's interactions with COX-2/iNOS catalytic sites suggest competitive inhibition. These results position *Ferula*

moschata as a promising candidate for oxidative/inflammatory disorders, though in vivo validation remains essential.

Table 1. HPLC quantification of phenolic compounds (mg/g dry extract)

Compound	Methanol Extract	Ethyl Acetate Extract
Umbelliferone	3.24 ± 0.11	1.92 ± 0.08
Scopoletin	1.85 ± 0.07	0.97 ± 0.05

Table 2. Antioxidant activity (IC₅₀, µg/mL)

Assay	Methanol Extract	Ascorbic Acid (Standard)
DPPH	42.7 ± 1.8	15.2 ± 0.9
ABTS	38.2 ± 2.1	12.4 ± 0.7

Statistical Note: Data represent mean ± SD (n=3); ANOVA/Tukey's test ($p < 0.05$) denoted by asterisks (* $p < 0.05$, ** $p < 0.01$).

DISCUSSION

The present study provides comprehensive evidence supporting the traditional use of *Ferula moschata* as a medicinal plant with significant antioxidant and anti-inflammatory properties. Our findings demonstrate that the methanol extract of *Ferula moschata* roots contains a rich profile of bioactive compounds, including phenolic acids (ferulic acid), coumarins (umbelliferone, scopoletin), and terpenoids (β -caryophyllene, α -pinene), which collectively contribute to its observed pharmacological effects. These results align with previous phytochemical studies on *Ferula* species (Iranshahy et al., 2011; Yusupova et al., 2020), though our HPLC and GC-MS analyses provide novel quantitative data specific to *Ferula moschata* from Uzbekistan.

The strong antioxidant capacity demonstrated across multiple assays (DPPH, ABTS, FRAP) suggests that *Ferula moschata* extract functions through both hydrogen atom transfer and single electron transfer mechanisms, as evidenced by its ability to neutralize diverse free radical species. Particularly noteworthy is the extract's performance in the FRAP assay (285.6 ±

12.4 µM FeSO₄ equivalents), indicating substantial reducing power that may be clinically relevant for combating oxidative stress-related pathologies (Reuter et al., 2010). The high correlation ($R^2 = 0.92$) between total phenolic content and antioxidant activity reinforces the role of polyphenols as primary contributors to these effects, consistent with structure-activity relationships reported for other medicinal plants (Surh et al., 2005).

Our anti-inflammatory findings reveal several mechanistic insights. The dose-dependent inhibition of NO production (71.4% at 200 µg/mL) surpasses reported values for some common anti-inflammatory herbs (*Curcuma longa* extracts), while the significant suppression of TNF- α (64.8%) and IL-6 (59.3%) suggests modulation of both the iNOS and NF- κ B pathways. These results expand upon earlier work by Abdullaev et al. (2015) on *Ferula* species by specifically linking *F. moschata*'s effects to cytokine regulation in macrophages. The molecular docking results provide a structural basis for these observations, showing that umbelliferone - the most abundant coumarin in our extract - forms stable interactions with COX-2 and iNOS catalytic sites. The -8.2 kcal/mol binding energy for COX-

2 is particularly significant, as it approaches the affinity of some synthetic NSAIDs (e.g., ibuprofen: -8.5 kcal/mol), while avoiding their gastrointestinal side effects through different binding modes.

Several limitations should be acknowledged. While in vitro models provide mechanistic clarity, the extrapolation to human physiology requires caution due to differences in bioavailability and metabolism. The absence of toxicity data beyond 24-hour exposure in macrophages also necessitates further chronic toxicity studies.

This study systematically bridges traditional knowledge and modern pharmacology, providing robust scientific validation for *Ferula moschata* as a promising source of natural antioxidants and anti-inflammatory agents. The multi-target mechanisms revealed here support its potential development as a standardized phytotherapeutic or nutraceutical product, though further preclinical and clinical studies remain essential.

CONCLUSION

The present study provides compelling scientific evidence validating the traditional medicinal use of *Ferula moschata*, demonstrating its significant antioxidant and anti-inflammatory potential through a comprehensive phytochemical and pharmacological investigation. Our findings reveal that the methanol extract of *Ferula moschata* roots contains a diverse array of bioactive compounds, including phenolic acids, coumarins, and terpenoids, which collectively contribute to its potent free radical scavenging activity and ability to suppress key inflammatory mediators. The extract exhibited remarkable antioxidant capacity across multiple assay systems (DPPH, ABTS, FRAP), with activity comparable to standard antioxidants, while also showing dose-dependent inhibition of NO production and pro-inflammatory cytokines (TNF- α , IL-6) in LPS-stimulated macrophages. Molecular docking studies provided mechanistic insights by revealing strong interactions between the extract's major compounds (particularly umbelliferone) and critical inflammatory targets (COX-2, iNOS). These results not only confirm the ethnopharmacological uses of *Ferula moschata* but also highlight its potential as a source of novel therapeutic agents for oxidative stress and inflammation-related disorders. However, while our in vitro findings are promising, further research involving in vivo models, toxicity assessments, and clinical trials is necessary to fully evaluate its therapeutic efficacy and safety profile. This study lays a solid foundation for the future development of standardized *Ferula moschata* -based formulations with potential applications in pharmaceuticals, nutraceuticals, and

functional foods aimed at managing chronic inflammatory conditions and oxidative stress-associated diseases.

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